SOME RELATIONS BETWEEN FERTILITY AND THE COMPOSITION OF THE DIET

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HAROLD M. HARSHAW, M.S.

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in the

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Some Relations Between Fertility and the Composition of the Diet

ABSTRACT.—When rations containing no known source of vitamin E were fed to albino rats, sterility resulted. Either our rations were contaminated with some unknown source of the antisterility factor, or this factor may be stored for long periods of time, for in occasional instances it was not until after 6 or 8 months that the females had become completely sterile. The use of wheat germ or wheat germ oil in the lard rations resulted in the birth of a normal number of litters, so our results are in harmony with the theory of Evans and Bishop that there is some previously unidentified factor necessary for normal reproduction. Our observations yielded no evidence that the sterility of our animals was due to anemia, or that vitamin E has any direct relation to the formation of hemoglobin. We have also examined a number of males which have received these various rations for different periods of time. Those males which were reared on lard diets, containing no vitamin E, were found to be sterile after from 65 to 100 days. The testes of these animals showed the typical condition of atrophy and lack of sperm, which has been described. On the other hand, when a source of vitamin E was provided in the ration of the males, they were found to be fertile in every case except one.

It is now less than four years since Evans and Bishop[1] announced the discovery of a new vitamin, which must be supplied if female rats are to be fertile. This substance is now commonly designated as vitamin E, though its existence is still questioned in some quarters. During the last three years numerous researches on this topic have been described, but in view of the fact that an excellent review of the literature has recently been published[2] we will limit ourselves to brief mention of a few of the more important points that are pertinent to the material we wish to present.

The first rations of Evans and collaborators contained 9 per cent of milk fat, and our initial diets contained 10 per cent of this substance. As described in an earlier paper[3], we failed completely at every point to confirm the results obtained by the California group, and this was also the experience of Nelson[4] and coworkers. We stated at the time that if vitamin E exists, it must be present in the milk fat we were using. We then changed our ration by substituting Crisco (hydrogenated cottonseed oil) for milk fat, and will merely say that we were unable to obtain any evidence of the existence of vitamin E while using that substance. Shortly after the change was made, however, we noted that according to Sure[5] cottonseed oil contains vitamin E. A similar report concerning Crisco was later made by Evans and Burr[6], and this has been confirmed recently by Kennedy and Palmer[7]. We at once substituted lard for Crisco, and we may say at once that since then our results have been in

almost complete agreement with those of Evans. We are now of the opinion that no evidence proving the non-existence of vitamin E can be obtained if rations are used which contain any significant quantity of milk fat, and in view of the work of Daniels and Hutton[8] we believe this is also true of skim milk powder. Nelson and collaborators[9] have recently reiterated that they have been unable to confirm the work of Evans. The rations they describe, however, contain 5 per cent of milk fat, and this may be the explanation of the discrepancy. In their most recent paper[10] a new suggestion is made that requires further study. In the present connection the most important statement is that desiccated rations are in some respect defective. In the more important feeding trials they used rations containing skim milk powder, and in addition yeast and cod liver oil were fed separately. Sterility was the rule on these rations, but if 5 per cent of water were added to the ration fertility resulted. We infer from their paper that 95 per cent alcohol, or wheat germ oil, might exert a similar protective action. We have had no experience with this procedure on which to base any comments, but we feel that if skim milk is used in a ration, any evidence indicating the non-existence of vitamin E requires careful confirmation. Our viewpoint receives some support in the failure of Sure[11] to confirm the observations of Anderegg and Nelson. A recent report by Mattill and Clayton[12] is in essential agreement with the findings we wish to present in this paper.

EXPERIMENTAL

We have followed essentially the same laboratory procedure as was described in a previous publication[3], so the details need not be repeated. An improvement has been introduced, however, by providing a steam sterilizer for the animal cages. It is probably superfluous to add that we have continued to use the white rat in our studies. We are omitting the observations made when Crisco was included in the diets, for these data were the same in character as when milk fat was used[3].

The first of our rations that contained lard, No. 304, was made up of casein* 20 per cent, corn starch 50 per cent, lard 10 per cent, cod liver oil 5 per cent, dried yeast 9 per cent, cellulose 2 per cent, salts 4 per cent, and was given to three groups of animals, Nos. 39, 40, and 49. Groups 39 and 40 were given an additional daily supply of vitamin B, in the form either of 0.5 gram yeast or 400 mgm. of the vitamin—Harris powder, for each animal. This was fed separately, and was continued from the time they were weaned. After they had been under observation

*We are omitting any extended description of the constituents of our diets, since most of them are in common use. The casein was a commercial preparation, but was extracted with some solvent before use. In some cases acidulated water was the only solvent, in others it was followed by alcohol and ether. Various salt mixtures were also used, but none of these modifications affected the final outcome.

26 weeks, this additional vitamin B carrier was also offered Group 40. The practice was also followed of taking vaginal smears of the females, beginning about six months after the animals were placed under observation.

No litters were born to the three females in Group 39 during the first four months of the trial, so thinking the male might be sterile, he was replaced by a stock male at the end of 19 weeks. Soon after this change, the females became pregnant and gave birth to 6 litters with 27 young in the next three months, but none of these was reared. Many of the young were born dead or died shortly after birth, and were undersized. One litter did live over three weeks, but even with extra vitamin B finally died. Subsequent to this there were five pregnancies, each of which ended in resorption. One female died, shortly after the birth of her first litter, leaving only two females in the group.

The results in Group 39 were practically duplicated by the females in Group 40. No young were born in the first months they were under observation, so at the end of 18 weeks the male was replaced because of the suspicion that he was sterile. This suspicion was later confirmed, both by breeding tests on stock females, and by a microscopic examination of a testicular smear when the animal was killed. This observation also is in accord with the reports of Evans[13], of Mason[14], and of Mattill and Clayton[12] that the males become sterile when limited to rations free of vitamin E. After the new male was introduced into this cage, eight litters were born containing 43 young. In most of these cases the young were born dead or were undersized and died in two or three days and in only one instance were the young brought to maturity. These litters were born during the first six months the animals were under observation. During the last four months the females became pregnant three times and gave the placental sign, but the embryos were resorbed. These facts are in accord with the statement of Evans[6] that vitamin E may be stored and for that reason fertility may persist for several months even though the animals may be maintained on a ration free of vitamin E. Fertility persisted longer in some of our animals than his data led us to expect, however.

Group 49 contained five females and one male, and their ration (No. 304) was the same as received by Cage 39, including the extra vitamin B fed separately. For some reason we are unable to explain, however, all the animals of this group grew slowly. They failed to become pregnant until long after the expected time, so after 23 weeks the male was killed and the testes examined. These were undersized, as shown in Table 5, and in addition they were watery and of a pulplike consistency. A stock male was then placed in this cage, and each female became pregnant once, but all the embryos were resorbed.

The next three groups, Nos. 41, 42, and 43, were started on Ration 322, which differed from those just described in that it contained potato starch instead of corn starch. This change was made because of the suggestion of Evans and Burr[15] that the potato tuber does not contain vitamin E. This ration contained 9 per cent yeast, but Groups 42 and 43 were given vitamin B in addition, to the amount of 400 mg. of the Harris yeast concentrate for each animal daily. The last 10 weeks of the experiment Groups 42 and 43 were given a diet containing 2 per cent of wheat germ oil, in an attempt to restore their fertility and thus confirm the report of Evans in this respect. Unfortunately the experiment was accidentally terminated before this was accomplished, when a fire killed all of our experimental animals.

The three females in Group 41 gave birth to three litters containing 12 young, though none survived, and one of these litters contained only one young rat, which was born dead. These females later became pregnant nine times, but resorption occurred in every instance.

The three females in Group 42 did only a little better than the one just described. Five litters were born, containing 25 young. Of these 21 were retained, but only eight were reared. After these young were born, there were four pregnancies, but in every case, the embryos were resorbed.

In Group 43, the four females gave birth to five litters of 25 young, but none of these was reared. In addition to this, the vaginal smears that were taken, beginning about four months after the beginning of the experiment, indicated that the females became pregnant in three cases, gave the placental sign at the proper time, but these embryos died later and were resorbed. After four or five months, it seemed probable that the males in these three cages were sterile, so they were killed and their testes examined. These were found to be undersized, their weights were below normal, and in two of the three cases, no sperm could be identified when the smears were examined under the microscope.

Up until the latter part of July, 1925, comparatively few young had been born to the groups receiving the lard rations, so a new group, No. 47, was started on Ration 335, containing wheat germ oil. Shortly after this feeding trial was started, however, it was demonstrated that the males in Groups 39 and 40 were sterile, and that the females were still capable of becoming pregnant. We decided, therefore, to expand our studies of rations of the type represented by No. 304. This ration, according to Evans, is free from vitamin E, and we decided to increase the use of rations of this type until it was decided whether or not females receiving them become sterile. Therefore, the ration of Group 47 was changed to No. 355, which differed from No. 304 only in containing a different salt mixture. Some three months after this change was made,

it seemed evident that the male was sterile, so he was removed. His testes were decidedly undersized, and no spermatozoa were found. After this male was replaced, no litters were born to the females in this cage, but the vaginal smears, taken after four months of the experiment had passed indicated that there were seven pregnancies, accompanied by the placental sign.

Four other groups, Nos. 50, 51, 52, and 53, were placed under observation on August 18, 1925. Groups 50 and 51 received Ration 356, similar to No. 304, except that it contained a different casein preparation. Group 51, however, was given 100 mg. of Harris' yeast vitamin daily, for each animal, and this amount was increased to 400 mg. when they reached maturity. Vaginal smears were taken daily, beginning the last of October. The males in these cages became sterile, as shown by examination, and were replaced by stock males on November 11.

No litters were born to the animals in Group 50, but after the male was replaced, there were seven pregnancies, each followed by resorption. In the case of Group 51, 5 resorptions were noted, after the stock male was placed in the cage. Two of these females gave birth to one litter each, the second week of January 1926, but these died the next day.

Groups 52 and 53 received Ration 357, very similar to those just described, but it contained only 6 per cent of dried yeast. Both groups, however, were given additional vitamin B concentrate, in the form of the Harris powder. For a time each animal was given 100 mg. daily, but at about the time maturity was attained, the quantity was increased to 400 mg. The females in these two groups were found to be pregnant in 10 instances, but in every case except one the embryos were resorbed, and in this instance the litter, born near the beginning of the experiment, died the next day. The males were killed on November 11, and their testes examined for sperm. The one in Cage 52 seemed still to be fertile but the other was apparently sterile.

In general, the results from these rations which contained lard confirm the reports of Evans. He found that females resorb their litters continually and only in rare instances are any young born. His males on this type of diet also become sterile in a short time, showing degeneration of the testes. Several litters were born to females on our lard diets, but many of them did not give birth to young. The vaginal smears from these animals, however, showed that resorptions following pregnancies were frequent, and in practically every case, the males also became sterile on these diets, nor were we able to correct these conditions by feeding the animals a large excess of vitamin B.

RATIONS CONTAINING WHEAT GERM OR WHEAT GERM OIL

As soon as evidence began to accumulate that females receiving the lard diets were becoming sterile, we decided to incorporate in our rations some material that had been described as containing vitamin E. Evans, as we have mentioned, found that wheat germ and wheat germ oil are rich in the substance which exerts a favorable influence on reproduction, and we turned to it in our attempts to find out whether such a substance would improve the rations we had been using.

One of the more significant of these trials was carried out with Groups 45 and 46, beginning May 23, 1925. There were four females in each group, and they received Ration 323, made up of casein, potato starch, lard, cod liver oil, dried yeast (9 per cent), salts, cellulose, and wheat germ oil. Nineteen litters were born to these females, with a total of 121 young. Of these 99 were retained, and 77 were reared. Two other litters were lost through accidents which had no relation to the method of feeding, and if these had not occurred we believe that the percentage of young reared would have been somewhat higher. However, the record of more than 77 per cent of the young reared, compares well with the record of our stock colony for the past two years. In all our groups of stock rats during this time, 75 per cent of the young were reared. In two of our stock cages, however, which received a ration containing dried ox liver, the females reared 95 per cent of their young, so it would appear that the results from Groups 45 and 46 still fall below the optimum. In order to facilitate comparisons we are including at this point records of young reared by our stock females.

TABLE 1.—REPRODUCTION RECORD OF STOCK FEMALES

Group	No. of Females	No. of Litters	Young Born	Young Retained	Young Reared	Per Cent Reared
63	4	27	188	149	86	58
65	3	17	102	88	67	76
1	5	17	98	85	68	80
2	4	12	89	68	41	60
71	2	6	39	29	20	70
A	2	6	44	35	31	88
B (a)	3	11	80	69	64	93
D (a)	4	10	81	64	62	97
F	3	8	26	22	18	82
Totals	30	114	747	609	457	75

(a) The ration of these groups was made up of yellow corn 72, dried liver 15, dried alfalfa leaves 10, cod liver oil 1, bone ash 2.

The final trial with wheat germ was made with Groups 54 and 55. Group 54 contained two females, while Group 55 contained three. All of these animals were from litters produced by the two preceding groups so their early history was the same. Both were given Ration 358, which

contained 9 per cent of wheat germ. The only difference in their treatment was that Group 55 was given an additional supply of vitamin B, fed separately. At first each animal received 100 mg. of the Harris concentrate daily, but this was increased to 400 mg. on October 10, 1925. The trial lasted until March 2, 1926, when the animals were destroyed by a fire.

During this period, comprising about four months of maturity, the females in Group 54 gave birth to five litters, with 29 young, but not a single one was reared. The results were decidedly better in the case of Group 55. It will be remembered that this group was given an additional supply of vitamin B concentrate amounting to 400 mg. daily, for each animal, from the time of maturity. This addition was undoubtedly responsible for the fact that a good proportion of the young born to the females in this group were successfully reared. Of the 57 young (10 litters) born, 49 were left with their mothers and 30 of these, or 66 per cent, were reared. Five of these young were lost through accidents, and had these not occurred, we believe that the percentage of young reared would have approached that of our stock colony. The fact that female rats have a high requirement for vitamin B during lactation has been noted by several observers[3, 16, 17]. As shown later, Figs. 6 and 7, two litters were resorbed, one from Group 46 and one from Group 54. We are unable to decide from our data whether or not any importance should be attached to that point, but an observation of Mattill and Clayton[12] may be significant in this connection. In the section of their paper "Curative Results With Female Animals," they noted that "On these synthetic rations there is a fairly linear relationship between the amount of vitamin E supplied and the promptness with which its functional result is achieved." Of 15 animals receiving a "low fat" diet containing 2 per cent of wheat germ oil, 11, or 74 per cent, gave a positive response. Their most important experimental ration contained 5 per cent of the oil. It may be that the quantity of wheat germ oil we supplied was below the optimum. However that may be, it is apparent from the results obobtained with these two groups, together with Groups 45 and 46, that the addition of wheat germ, or wheat germ oil, to our basal ration brings about a vast improvement in the reproductive records of female rats.

It is, however, necessary to include a liberal supply of vitamin B in the ration, in order that the animals may rear their young successfully. The rat apparently requires not only vitamin E for normal reproduction, but also needs an increase in the supply of vitamin B for normal lactation and the successful rearing of young. In the case of Groups 45 and 46, this additional vitamin B was supplied in the form of 9 per cent of dried

yeast, an amount which is considerably in excess of that required for normal growth.

A summary of our records is presented in Table 2 and in Table 3, the composition of the rations is given. Occasional minor changes were made in the diets of some groups, but as the results were negative, we are omitting the details.

TABLE 2.—OBSERVATIONS MADE ON REPRODUCTIVE CYCLE OF FEMALE RATS

Ration No.	Group No.	Age at Last Observation Days	No. of Females	Inseminations	Litters	Resorptions	Young Born	Young retained	Young reared
A	On diets supposedly free of vitamin E.								
	39	361	2	12	5	6	27	26	0
304	40	385	3	13	8	3	43	40	3
	49	237	5	10	0	5	0	0	0
	47	268	4	10	0	7	0	0	0
	41	322	3	11	2	9	12	11	0
322	42	324	3	11	5	4	27	21	8
	43	328	3	15	5	3	25	23	0
356	50	224	4	10	0	9	0	0	0
	51	218	3	9	2	6	9	9	0
357	52	225	3	6	1	5	4	4	0
	53	226	3	7	0	2	0	0	0
Totals			36	114	28	59	167	134	11
B	On Diets Believed to Contain Vitamin E								
323	45	317	4	12	12	0	85	68	48
	46	331	4	10	9	1	52	47	34
358	54	220	2	7	5	1	29	26	0
	55	229	3	10	10	0	57	49	25
Totals			13	39	36	2	223	190	107

TABLE 3.—COMPOSITION OF RATIONS*.

Ration Number	304	322	323	335	357	358
Casein	20	20	20	20	20	20
Corn Starch	50		49	50	53	50
Potato Starch		49				
Crisco				8		
Lard	10	10	8		10	10
Cod Liver Oil	5	5	5	5	5	5
Cellulose	2	3	3	2	2	2
Salts	4	4	4	4	4	4
Yeast	9	9	9	9	6	
Wheat Germ						9
Wheat Germ Oil			2	2		

*Some of the rations used are not listed in this table, as they differed in minor details only. These diets are described more completely in the text.

It is at once evident that the animals receiving wheat germ, or wheat germ oil, were much more fertile than those that did not. The former group produced 46 litters, and our observations indicated that two were resorbed. The females that did not receive vitamin E produced 28 litters and resorbed 59. The contrast becomes all the greater when we remember that the former group contained 13 females, and the latter 36. Another point we believe worthy of mention is the large number of inseminations that did not result in pregnancy. It is of course obvious that our records on this point represent a minimum, for we sometimes noted pregnancy and failed to note the insemination that had occurred. However, in order to obtain the total on which our calculations are based, we added inseminations not followed by pregnancy to the total number of known pregnancies. Among those receiving the basal ration 114 inseminations could be accounted for, but of those 28, or nearly 25 per cent, failed to result in pregnancy. In a similar way 49 inseminations could be accounted for among those receiving rations containing vitamin E, and only one failed to result in pregnancy. No inseminations were recorded unless spermatozoa could be identified in the vaginal smear. It is evident, however, that failure to subsequently become pregnant does not necessarily indicate any abnormality of the female. The males receiving the basal diets ultimately became sterile, and according to Evans[13] there is a stage during the process when abnormal spermatozoa are formed.

It is also evident from Figs. 1 and 2 that sterility of the females was not always as immediate and complete as the literature might lead one to expect. In fact, some of the females on diets that produce sterility, gave birth to three litters; and, in some cases, a litter was born seven months after they were placed on the experimental diet. Evans and Burr[6] have reported that fertility may persist for three or four months.

THE RELATION BETWEEN REPRODUCTION AND THE NUMBER OF ERYTHROCYTES IN THE BLOOD

The experiments described in this section were carried out because of the suggestion of Hart and colleagues[18] that vitamin E may be an organic substance containing the pyrrole nucleus, which exerts a favorable influence on reproduction by preventing or curing anemia. Since there is some evidence[19,20] that yeast contains the pyrrole nucleus in demonstrable quantities, we hesitated to accept this theory, but decided to use some of our animals then under observation for preliminary tests. These consisted in making counts of the red blood cells, and in making comparative estimations of the hemoglobin content of the blood. The counts

were made with an American Standard Hemocytometer, using a Levy counting chamber, and the hemoglobin readings were made with a Dare hemoglobinometer. Some of the animals were well supplied with vitamin E, while others supposedly received none of that substance, and were sterile. These estimations were made at various stages in the oestrus cycle, as determined by the vaginal smears, and also at various stages of gestation and lactation. A number of males were also included, some receiving the stock ration, while others received the synthetic diets, complete in some cases, in others free from known sources of vitamin E. Some of the rations were also changed so as to include hemoglobin.

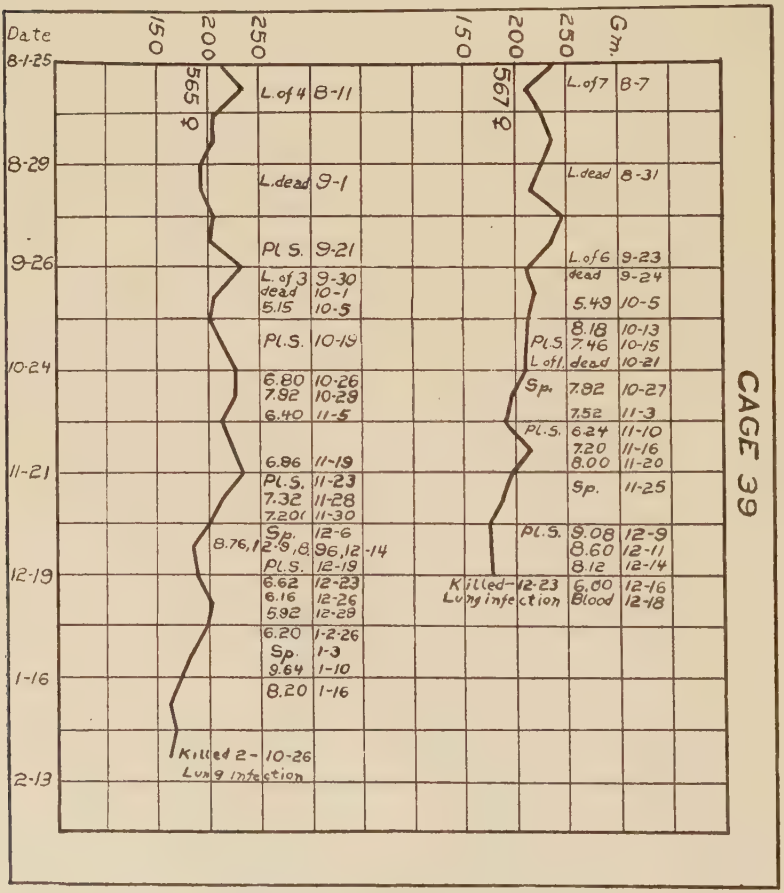

Fig 1.—Group 39, placed under observation March 21, 1925. Ration 304; in addition each animal received 0.5 gram yeast daily until June 18, when this was replaced by 0.4 gram of a vitamin B concentrate. On October 10, one half the casein, 10 per cent of the ration, was replaced by hemoglobin.

It seemed probable that this material would supply any special chemical groups required for the formation of hemoglobin and red blood cells, and if the cases of nutritional sterility we had observed were due to anemia, these animals should afford some evidence of the fact. The more important parts of our data are presented in Figs. 1-8; some of the less significant charts are omitted in order to conserve space.

EXPLANATION OF CHARTS

Figs. 1 to 8 include the periods when the animals were practically mature. Vaginal smears were taken daily of the females of these groups, and the dates of insemination are indicated, as well as those of the pla-

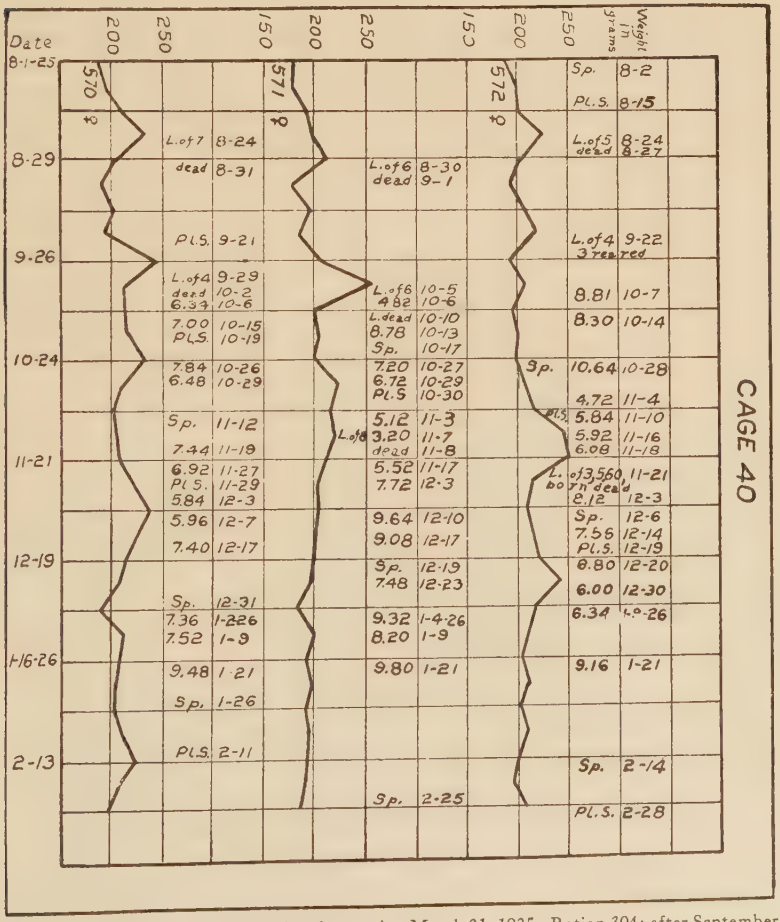

Fig. 2.—Group 40, placed under observation March 21, 1925. Ration 304; after September 23, each animal was given in addition 0.4 gram of a vitamin B concentrate.

cental sign. When blood counts were taken, they are also indicated. The following example will probably make clear the meaning of the symbols employed.

Sp. indicates that sperm were found in the vaginal smear.

Pl. S. indicates the placental sign.

The figures such as 7.92 indicate the count of red cells in the blood in millions per cubic millimeter.

The figures such as 1/2/26 indicate the date in month, day and year.

L is used as an abbreviation for Litter.

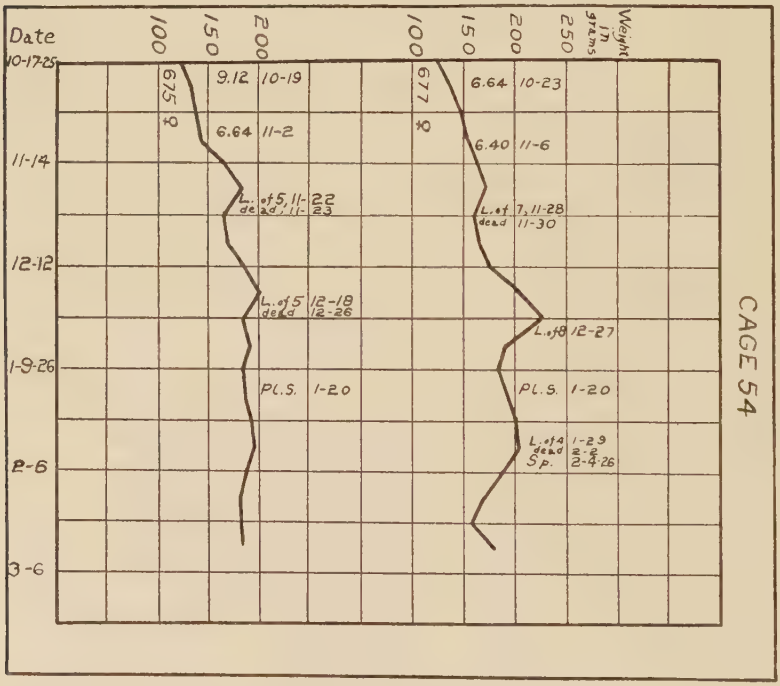

Fig. 3.—Group 42, placed under observation May 16, 1925. Ration 322; after July 3 additional vitamin B was supplied separately.

The charts show, as would be expected, that there is a strong tendency for the blood count to decline during the latter stages of pregnancy, with a marked drop at the time of parturition, followed by a gradual return to normal. Most of these counts, when not influenced by pregnancy, are little if any below the normal. Our determinations on stock animals varied between 8 and 9 million per cubic millimeter, while Donaldson[21] reports an average of 8.8 million from counts made on 50 normal rats. Scott[22] likewise reports an average count of 8.8 million from observations on 12 normal animals, and Jackson and Riggs[23] observed

between 8 and 9 million. In general, the estimations made on the hemoglobin content of the blood varied with the red cell counts.

The feeding of a ration containing hemoglobin had little or no effect on the red cell count, and there was no apparent effect on the ability of the rats to reproduce. This is illustrated especially by Groups 39 and 47 which, for a considerable period of time, received hemoglobin in the diet. The females in these cages had, according to their smears, been resorbing their embryos when they became pregnant, and this

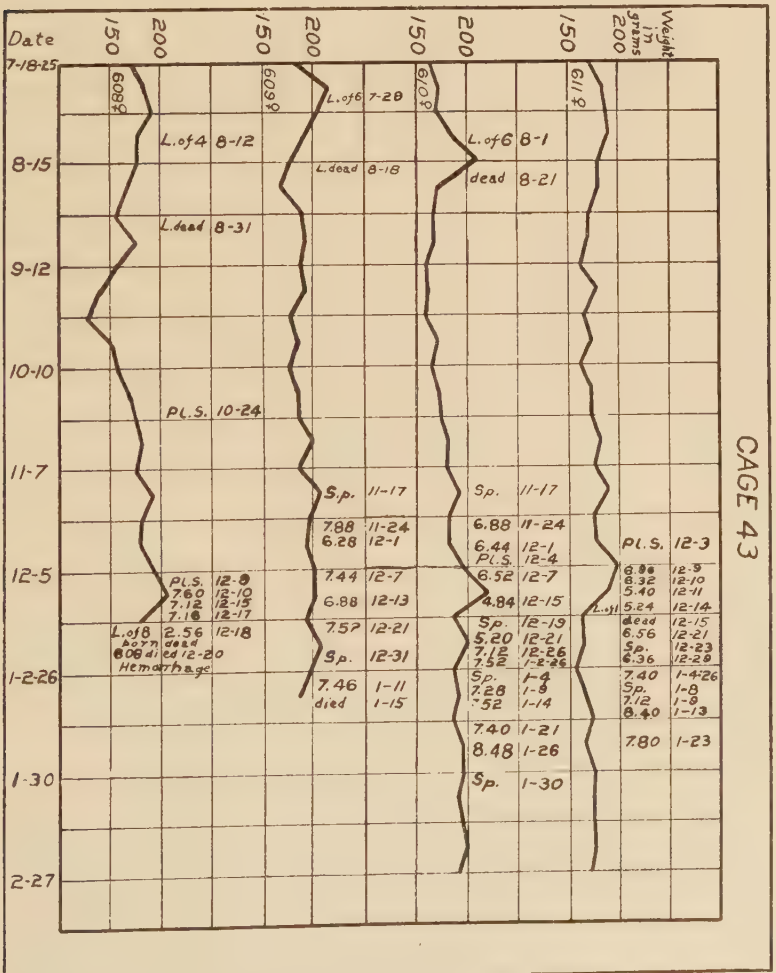

Fig. 4.—Group 43, placed under observation May 16, 1925. Ration 322; on December 19, the ration was changed by including 2 per cent of wheat germ oil, which replaced an equal quantity of lard.

condition was continued after they were placed on the hemoglobin diet. Another fact which tends to indicate that anemia on these rations does not interfere with reproduction is noted in connection with Groups 45, 46, 54, and 55. The erythrocyte counts of these females were lower, if anything, than of those just mentioned, and yet their reproduction record was quite satisfactory. It would seem, therefore, that anemia is not a factor in the failure of reproduction when animals receive rations free from vitamin E.

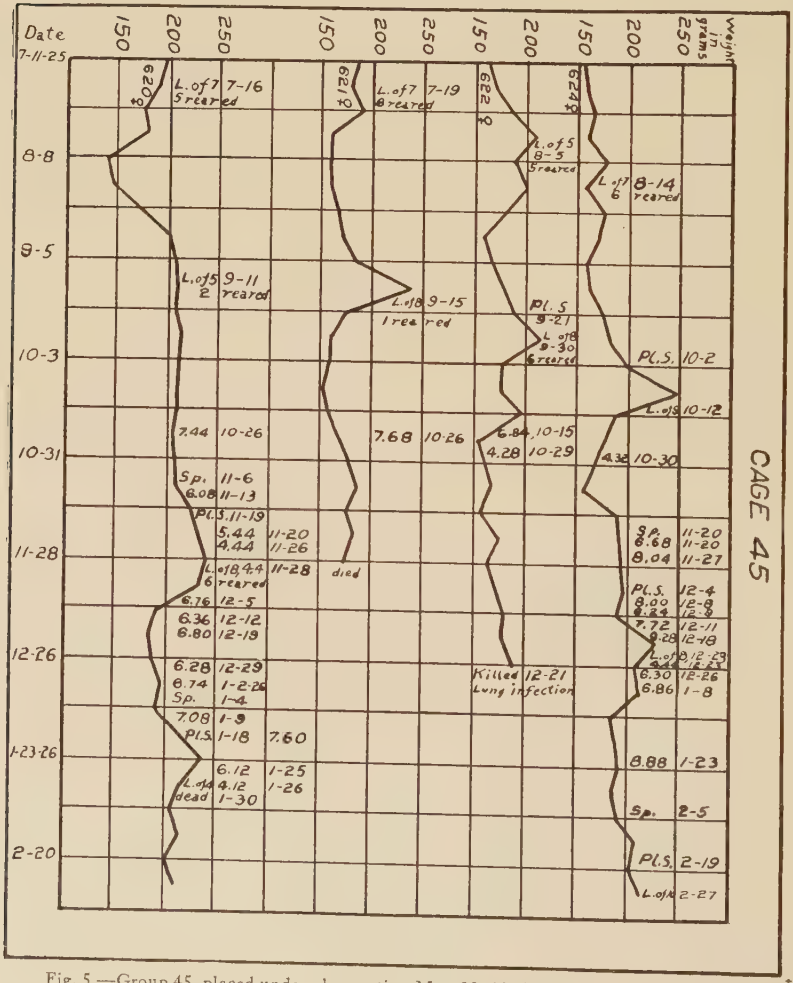

Fig. 5.—Group 45, placed under observation May 23, 1925. Ration 323 (containing wheat germ oil).

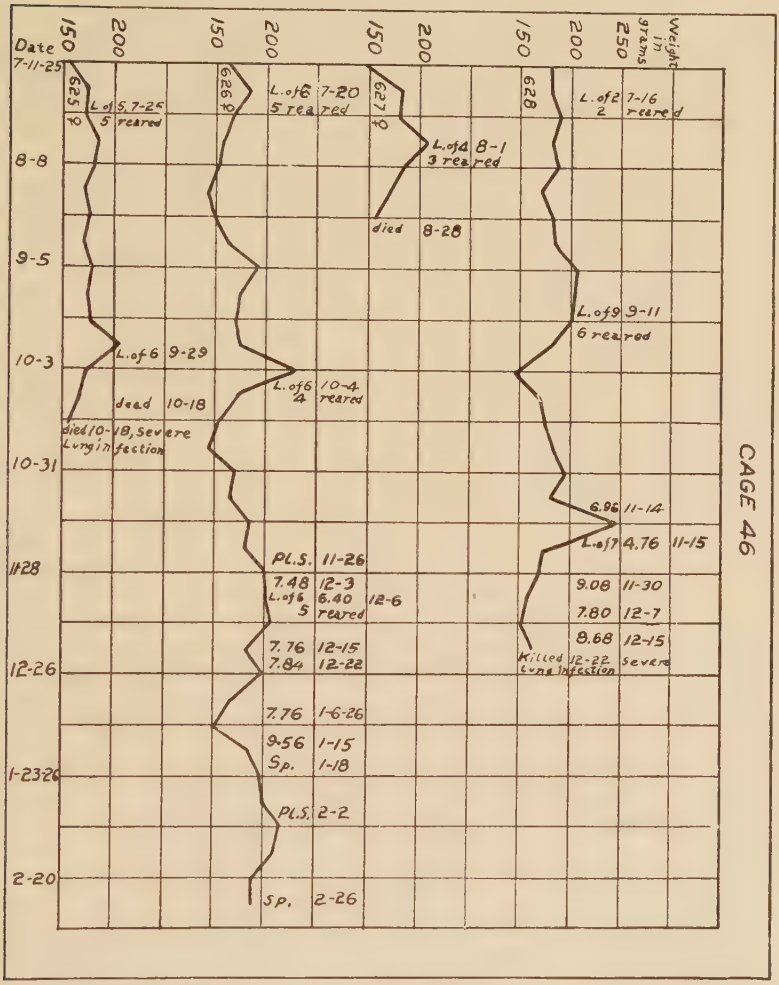

Fig. 6.—Group 46, placed under observation May 23, 1925. Ration 323 (containing wheat germ oil); additional vitamin B was supplied separately.

Of the animals that supposedly received diets containing vitamin E, Groups 16 and 34 were under observation before we began taking vaginal smears, and for that reason have not been mentioned thus far. Since they failed however to yield any positive evidence of the existence of vitamin E, we feel that they should not be omitted. Such data as we have are given in Table 4.

Since we did not take vaginal smears of these animals, it is impossible to explain the failures to bear young. It will be noted that the failures

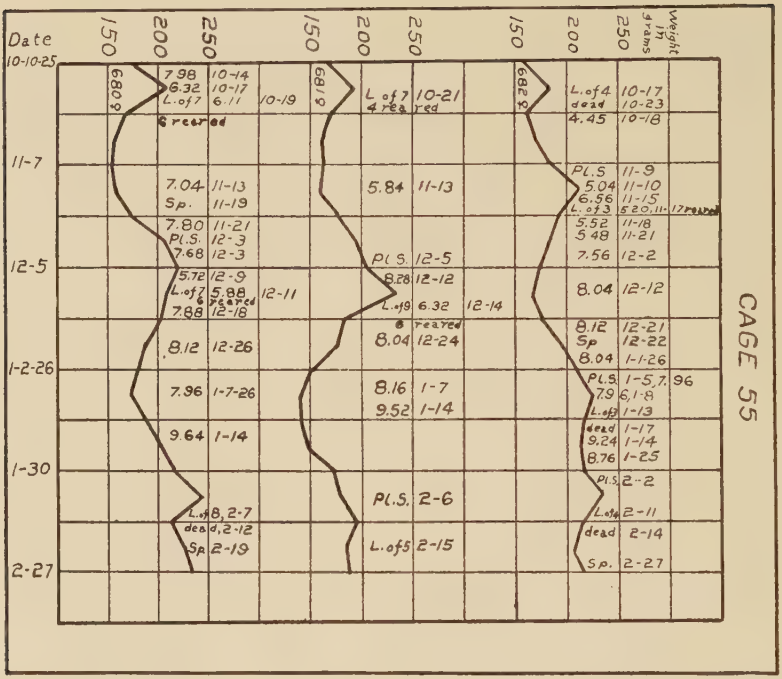

Fig. 7.—Group 54, placed under observation August 18, 1925. Ration 358 (containing wheat germ.)

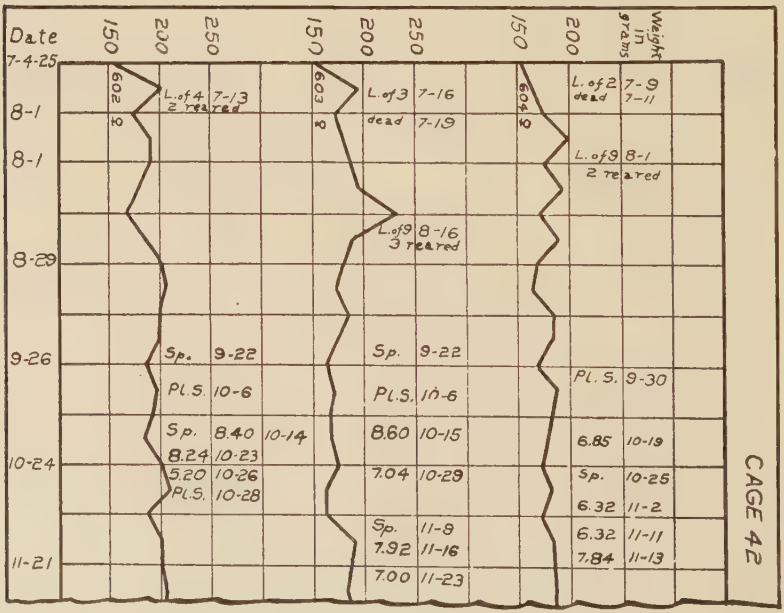

Fig. 8.—Group 55, placed under observation August 18, 1925. Ration 358 (containing wheat germ); additional vitamin B was supplied separately.

were especially conspicuous in Group 34. We did determine by functional test that the male was fertile at least as late as August 14, 1925. Additional data on this animal are given in Table 5. It seems very certain then that the failures to reproduce must be ascribed to the females but from the data at hand we do not attach much significance to the fact. We have observed over 125 females, in 37 different groups that received similar diets, but Group 34 is the only one that made a near approach to complete sterility.

TABLE 4.—REPRODUCTIVE RECORD OF 2 MISCELLANEOUS GROUPS ON RATIONS THAT PRESUMABLY CONTAIN VITAMIN E.

Group 16 Ration (192)[a]					Group 34 Ration (209)[b]		
Animal 379	Animal 380	Animal 382	Animal 406	Animal 426	Animal 498	Animal 512	Animal 513
B3-28-24[c] 0 5-3[d] L of 7 8/2/24 None reared L of 9 10/14 6 reared	B3-29-24 0 5-3 L of 3 7/19/24 L dead 7/20 L of 3 8/31 2 reared L of 7 11/19 6 reared	B3-24-24 0 5-3 L of 4 5/4/24 None reared L of 7 10/11 None reared L of 8 12/11 None reared L of 9 1/2/24 None reared L of 4 2/10 3 reared	B8-31-24 0 9-30 L of 7 13/18/24 None reared L of 6 1/19/25 None reared	B8-31-24 0 9-30 L of 3 1/30/25 None reared	B12-13-24 0 1-17-250	B12-29-24 2-7-25 L (?)[e] 8/28/20	B12-29-24 0 2-7-25
Discontinued 5-16-1925					Discontinued 10-12-1925		

(a) Casein 20, corn starch 53, Crisco 10, Cod liver oil 5, yeast 6, salts 4, cellulose 2. The ether and alcohol extracts of 20 parts of wheat germ were added to the above materials.

(b) Casein 20, starch 50, Crisco 10, Cod liver oil 5, wheat germ 9, salts 4, cellulose 2.

(c) Date of birth.

(d) Date placed under observation.

(e) One young, partly eaten, was observed.

THE INFLUENCE OF DIET ON THE FERTILITY OF MALES

In several cases it seemed that the failure of females to become pregnant was due to the sterility of the male, so a number of the latter were killed, and the testes examined. In order to secure a larger number of males for examination, additional groups were given experimental diets, some of which contained vitamin E, while others contained no known source of this substance. In most cases this examination included the taking of the weights of the testes, a search for sperm in a smear taken from the cut surface, and with a few exceptions the structure of the testes was also investigated* microscopically. Our observations agree in all respects with the excellent description of Mason[14].

So far as indicating the existence of vitamin E is concerned, the males yielded even more striking evidence than did the females. Without exception, males reared on diets containing wheat germ, or wheat germ oil, had testes of normal weight, and numerous sperm could be identified in the testicular smear. If, however, the rations were free from vitamin E, degenerations of the testes was the almost invariable rule. If sufficient time were allowed on such a diet, the testes became soft and watery in practically every case, and no sperm could be identified in the testicular smears. Apparently 65 to 90 days on such diets is the time required to produce sterility, for at that time the testes were practically always undersized, and at best the sperm were few in number.

The exceptions may be noted in Table 5. Rat 454 received a ration that is presumably rich in vitamin E for 135 days after weaning, and was then transferred to Ration 304. The animal was killed 131 days later, and the testes examined. These organs were normal in weight, and numerous spermatozoa were identified in a smear taken from the cut surface. Mattill and Clayton[12], however, state that if vitamin E is generously supplied for the first 90 days of life, early sterility of males is not to be expected, even though the vitamin is later removed from the diet. Possibly we should state that all animals in Section A, except No. 454 were placed on the deficient diets at weaning time.

Some of the males were given greatly increased quantities of vitamin B, 400 mg. daily of the Harris concentrate, but this was ineffective in preventing this type of sterility. A summary of our observations is given in Table 5.

In addition to the animals listed in Table 5, one more, No. 463, should be mentioned. This male was born October 17, 1924, and on

*We are greatly indebted to Dr. F. F. McKenzie for the valuable assistance he so cheerfully contributed in preparing and interpreting the sections.

November 15 was placed on Ration 235. This ration contained 10 per cent of Crisco, and presumably supplied a considerable quantity of vitamin E. One of the females in this group gave birth to a litter on July 21, 1925, so it seems this male must have been fertile at least as late as July 1. On July 11 his ration was changed by substituting 2 per cent of wheat germ oil for an equal quantity of Crisco, but there were no further pregnancies in this group. The male was killed on October 2, 1925, and was found to be sterile. No sperm could be found in a testicular smear and microscopic examination of the tissue revealed an advanced state of degeneration.

TABLE 5.—RELATION OF VITAMIN E TO FERTILITY OF MALES

Group No.	Rat No.	Ration No.	Days on Ration	Weight of one Testicle (gms.)	Spermatozoa
A	Diets free of known sources of vitamin E.				
48a	649	355b	96	0.97	Numerous
48a	650	355b	126	0.45	none
26a	454	304	131c	1.37	numerous
39	568	304	137	0.54	none
40	569	304	150	0.50	none
40	588	304	149	0.68	none
49	656	304	154	0.47	none
41	599	322	94	0.55	----
41	601	322	109	0.40	none
42	605	322	181	0.51	none
43	613	322	181	0.83	none
47	634	355b	104	0.50	none
50	662	360d	89	0.46	none
51	666	360	89	0.54	none
51	696	360	65	1.10	few
52	670	357	89	1.15	numerous
53	674	357	89	0.94	none
53	698	359e	63	0.65	none
58	691	357	56	1.24	numerous
58	694	357	85	0.45	none
58	693	357	115	0.53	none
MeB	Diets contained wheat germ or wheat germ oil				
34a	496	209f	272	1.28	numerous
56	686	358	57	0.96	numerous
56	687	358	86	0.91	numerous
56	679	358	116	1.17	numerous
57	688	358	56	1.01	numerous
57	689	358	115	1.05	numerous

(a) This group is not described in this paper.

(b) Ration 355 is identical with No. 304, except a different salt mixture was used.

(c) This animal was mature when placed on the experimental diet, and this may explain the fact that fertility persisted so long.

(d) Ration 360 is identical with No. 355 except a different salt mixture was used.

(e) Ration 359 is identical with No. 357 except a different casein preparation was used.

(f) Ration 209 contained both Crisco (10 per cent), and wheat germ (9 per cent).

SUMMARY

When rations containing no known source of vitamin E were fed to albino rats, sterility resulted.

Either our rations were contaminated with some unknown source of antisterility factor, or this factor may be stored for long periods of time, for in occasional instances it was not until after 6 or 8 months that the females had become completely sterile.

The use of wheat germ or wheat germ oil in the lard rations resulted in the birth of a normal number of litters, so our results are in harmony with the theory of Evans and Bishop that there is some previously unidentified factor necessary for normal reproduction.

Our observations yielded no evidence that the sterility of our animals was due to anemia, or that vitamin E has any direct relation to the formation of hemoglobin.

We have also examined a number of males which have received these various rations for different periods of time. Those males which were reared on lard diets, containing no vitamin E, were found to be sterile after from 65 to 100 days. These testes of these animals showed the typical condition of atrophy and lack of sperm, which has been described. On the other hand, when a source of vitamin E was provided in the ration of the males, they were found to be fertile in every case except one.

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